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BULLETIN

OF THE

TORREY BOTANICAL CLUB

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Vol. 51 No. 12

BULLETIN
OF THE
TORREY BOTANICAL CLUB

DECEMBER, 1924

A preliminary account of the influence of light and temperature on growth and reproduction in Chara fragilis*

JOHN S. KARLING

(WITH PLATES 11–13)

The fruiting in nature of the different species of the Characeae is reported by Migula (25), Allen (3), and Robinson (30) to occur during the summer and early fall. In undertaking a cytological study of nuclear and cell division and fertilization in the Characeae three cultures of *Chara fragilis* Desvaux were placed on an east window-sill in the laboratory on September 24, 1923, to determine whether the stimulus of higher temperature and more favorable illumination would lead to the development of reproductive organs at other than normal times. The vegetative growth of these cultures was vigorous, but no antheridia and oogonia were formed. In view of results recently described by Garner and Allard (17), Adams (1), and Harvey (19) it was thought worth while to test the cultures as to photoperiodism. Consequently on January 8, 1924, as a preliminary experiment, two of the cultures were placed under a 60-watt 120-volt electric light suspended 15 cm. above them and given continuous illumination throughout the night. Within a few days antheridia and oogonia appeared in abundance on these plants, while the control culture remained entirely free from reproductive organs. It was then determined to make a further preliminary study of the response of *Chara fragilis* to changes in illumination periods and temperature with the particular purpose of obtaining material for cytological study. The

* Contributions from the Department of Botany of Columbia University, no. 337.

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results so far obtained in these experiments are here reported. A further, more extensive series of experiments is now being made with controlled temperature and light intensities.

The problem of the periodicities in the life cycles of the algae has been widely studied both in the field and under laboratory conditions. The literature in this field is extensive, and I shall summarize only those results that deal more directly with the problem of photoperiodism as related to sexual reproduction. Klebs (23) aimed to demonstrate complete environmental control over the life cycles of various algae and fungi which he studied, but his results have not been generally confirmed. Many of his experiments involve environmental conditions which are scarcely ever realized in nature and are hardly applicable to the explanation of the normal periodicity of algae. Similar studies by a large number of investigators both in the field and laboratory have yielded diverse results and consequent views regarding the underlying causes of algal periodicities. The results of these studies, however, cannot be said to have been decisive either for the internal or environmental periodicity theories. Danforth (13), Copeland (11), Hoyt (22), Williams (35), and Transeau (31) hold that the periodic changes in the life cycles of the algae that they studied resulted not so much from external as from internal conditions and hereditary tendencies. Comère (10), Benecke (7), Fritsch and Rich (16), West and West (34), Brown (9), and Hodgetts (21), on the other hand, take the opposite view and account for the periodic changes on the basis of change in external environment.

For the higher plants many observers, including Corbett (12), Bailey (4), Rane (28), Bonnier (8), and Vöchting (32), have held that increased illumination led to rapid growth and to early production of flowers and seed. Results recently described by Garner and Allard (17), Hendricks and Harvey (20), and Adams (1) relating to a wide variety of flowering plants show that the relative length of the day is a primary factor in growth and development, and particularly in determining the occurrence of sexual reproduction.

Relatively few investigations have been made on the response of Characeae to light changes. In 1894, Richter (29), using *Chara fragilis* and *C. hispida*, found that the shoots were positive and the rhizoids negative in their response to light. In cultures

of *C. hispida* grown a meter or more from a window, Müller (26) found plants in which some of the internodes were without corticating cells. He also grew cultures of *C. foetida* in dark and half-dark rooms and in full daylight. In many of the plants grown in reduced illumination the corticating cells were either absent or abnormally arranged about the internodes. In such cases, the corticating cells, instead of adhering closely to the internodal cell, formed a loose sheath. Müller, however, made no experiments involving the lengthening or shortening of the period of illumination.

Nonweiler (27) grew *Chara strigosa* with peat and sand as substrata in sodium chloride solutions of various concentrations in full and reduced illumination. Increased concentration of the solution reduced the length but increased the diameter of the internodes. He observed that high temperature and light intensity hindered length growth. Ernst (14) reports that the length of the oospores of *Chara crinita* varied considerably with changes in temperature and light intensity, culture medium, and substratum on which the cultures were grown. Cultures grown under double-walled bell-jars with potassium bichromate and with ammoniacal cuprous oxide solution, or in reduced illumination, produced shorter oospores than in normal cultures. Hodgetts (21) in his study of the periodicity of fresh-water algae in nature reports that *Nitella flexilis* develops most rapidly when the temperature is moderately high, and that abundant bright sunshine is very necessary for oospore-production.

CHARA FRAGILIS GROWING IN NATURE

The plants used in the following experiments were secured from a small, shallow pond in Van Cortlandt Park, New York City, during the interval between September, 1923 and April, 1924. This is apparently an unreported locality for *Chara* in the vicinity of New York City. The water in this pond during winter of 1923 and the early summer of 1924 was shallow (5–20 cm. deep) and during June and July when the vegetative growth appeared most vigorous, the plants did not attain a length over 45 cm. The growth from October to March was negligible. It seems probable that when the winter is severe the tops of the plants may be killed by freezing. In such an instance, the nodes and other parts of the plants buried in the soil give rise to the next year's growth.

In the mild winter of 1923–24 the plants did not die down but remained alive under the ice when the pond was frozen over. Healthy green plants were secured throughout the entire winter. On several visits to the pond it was frozen over with a thin sheet of ice, and when the ice was broken away the plants were found to be in a dormant, healthy condition with a deep green color. The temperature under the ice was approximately 2° C. The plants at that time were only about 15 cm. in length, including the dead basal end. The internodes were 13 to 15 mm. in length and .75 mm. in diameter. The leaves and main axis were covered with brownish-colored plankton forms.

The formation of antheridia and oogonia in *Chara fragilis*, according to Migula (25) and Robinson (30), occurs generally from July until September. When this bed of *Chara* was discovered on September 19, 1923, there were no indications of reproductive organs. The plants were examined carefully throughout the winter for the appearance of these structures The first antheridia and oogonia occurring spontaneously were observed on May 26, 1924. The plants were still partly covered with brownish plankton, but they had increased several inches in length and branched freely.

EXPERIMENTAL METHODS

Large masses of plants were brought into the laboratory and grown in shallow pots with sand loam as a substratum and placed in battery jars. The battery jars were of various sizes, ranging from 15 to 30 cm. in diameter and 30 to 45 cm. in depth. The taller jars were found to be more favorable than the smaller ones. Tap water was used in every case. Very little difficulty was encountered from invasion of algae. A small crustacean was introduced into the jars to keep down the growth of the algae whenever they became abundant. When necessary the pots were lifted out and the jars were cleaned. Fresh water was added once or twice every month.

The cultures were placed in north, east, south and west windows in the laboratory where they received varying degrees of illumination. All cultures with the exception of those in the north windows received the direct rays of the sun several hours each day. The reponse to this increase in illumination and temperature was immediate in every culture. The plants began

to grow vigorously and branch freely. Two cultures of the plants and a control were used in each of these preliminary experiments. The plants were allowed to acquire a good vegetative development by leaving them in their positions for several days to weeks before they were subjected to experimental conditions. For increasing the period of illumination a 60-watt 120-volt mazda lamp was placed approximately 8 cm. above the battery jars. In every case the light bulbs were immersed in a battery jar of water to prevent an increase in temperature when they were burning. In all experiments the light was turned on at sunset; and the daylight period was reckoned from sunrise to sunset. The intensity of illumination when the sun was not shining directly on the cultures was measured rather roughly each week with a foot-candle meter. The controls in every case were covered with blackened bell-jars at sunset. The electric light was used only to supplement the normal daylight of the period of each experiment, and was not turned on until sunset. At the time that *Chara fragilis* began to fruit out-of-doors on May 26, 1924, the period of daylight had increased more than five hours over that in January when the experiments were begun. Thus the last cultures studied were receiving relatively more daylight and less electric illumination. The increase of the daylight period is shown in CHART 2.

A thermometer was placed in each battery jar, and temperature readings were made at intervals of four hours. Each culture was examined daily with a hand lens, and when there were indications of antheridia and oogonia the plants were examined with the low power of a microscope to determine with certainty the presence of reproductive organs. Photographs were made as soon as the first antheridia and oogonia had reached full size. Representative plants from each culture were selected and photographed. Records of the cultures are here reported, covering the period from October 18, 1923 to November 6, 1924.

OBSERVATIONS AND RESULTS

SERIES 0, CULTURES 0–1 AND 0–2

Collected in park pond.....Culture 0–1, Oct. 18; Culture 0–2, Dec. 28
Outside on east window-sill.....Oct. 18 to Jan. 11
Inside on shelf before east window with all-night electric illumination
Jan. 11 to March 26
On shelf in east window with only daylight illumination. . March 26 to Nov. 6

SERIES 0, CULTURE 0–3

Collected	1922
Grown in greenhouse	1922 to Jan. 22, 1924
Grown in greenhouse under all-night electric illumination	Jan. 22 to April 23
Grown in north window of laboratory with only daylight illumination	April 23 to Nov. 6

Series 0, Culture 0–1 consisted of three jars with a control. The plants were brought from the field on October 18 and grown outside on an east window-sill. The plants were there subject to alternate chilling and warming as the weather changed. On bright days the morning sunlight fell directly on the cultures. The plants were green and healthy but grew slowly. They had increased about three inches in length when on January 11 the culture was placed on a shelf inside of the window under electric illumination during the night. A more vigorous growth was immediately evident. The internodes increased in length, and the leaves spread out at right angles to the main axis. As the experiment continued there was a noticeable difference in growth as compared with the control. The control plants were shorter and stockier and deeper green in color. On January 21 antheridia and oogonia appeared, ten days after the all-night illumination was begun. There was no indication of fruiting in the control. The electric illumination was discontinued on March 8. Photographs were made on March 26. The development of antheridia and oogonia was slow in comparison with that in some of the later cultures. FIG. 1, PLATE 11 shows the result of the continuous illumination in contrast with the condition of the control culture shown in FIG. 4, PLATE 11. When the electric illumination was discontinued, the culture with its control was left in the same place in the east window. It continued to grow and fruit moderately throughout the summer, but by September 12 the whole culture was dead except for the tips of the plants. A few antheridia and oogonia were present in the two upper whorls of leaves. The tips, 10 cm. long, were transplanted to a new pot of sand loam where they subsequently began a rapid vegetative growth. By October 19 the plants were approximately 25 cm. in length, but no reproductive organs were present on the new growth. Since that time, until November 6, a few sporadic antheridia and oogonia have been formed on the upper whorls of leaves.

The control culture began to fruit on June 7. The fruiting continued throughout the summer, and on September 12 tips

10 cm. long were transplanted to a pot of fresh sand and loam. A good vegetative growth followed, and on October 13 a few antheridia and oogonia were observed on this new growth. By November 6, however, these had fallen off.

The plants of Series 0, Culture 0–2, consisting of a single jar, were brought in from the park on December 28, 1923 and grown in the east window alongside Culture 0–1. Its growth, however, had not been as vigorous when on January 18 it was placed inside of the window under the same light and temperature conditions as 0–1. Antheridia and oogonia were formed twenty-one days later, on February 9. No reproductive organs were present in the control culture at that time. Culture 0–2 continued to fruit during the summer, but the plants were very slender with long internodes and short leaves. On September 12 a number of 10 cm.-long tips of these plants were transplanted. Growth was very slow, and by the first of November the entire culture was dead. The original culture, pruned down on September 12, began to grow rapidly, and on October 19 the new growth was approximately 8.5 cm. long. Antheridia and oogonia were abundant on the new growth. By November 6, the longest plants were about 25 cm. long, with a few reproductive organs in the apical whorl of leaves.

Series 0, Culture 0–3, consisting of two jars, contained about seventy-five plants of *Chara fragilis* which were collected in 1922 and had been growing in the greenhouse for two years without forming reproductive organs. This culture had been subject to usual greenhouse conditions in a house used for ordinary herbaceous annuals. On January 22, 1924, the jars were placed under a 75-watt 120-volt light suspended 1 meter above. The light was kept burning throughout the entire night. On March 14, fifty days later, antheridia and oogonia appeared in the two uppermost leaf whorls of the plants. The night temperatures in the greenhouse were considerably lower than in the laboratory in which Cultures 0–1 and 0–2 were grown. FIG. 2, PLATE 11 shows this culture in contrast with its control. Nearly every oogonium formed in this culture developed mature oospores, while from the thousands of antheridia and oogonia formed in 0–1 and 0–2, less than twenty-five mature oospores were developed. In these latter cultures the oogonia turned white in color and dropped off after attaining full size.

Culture 0–3 continued to fruit until July. By September 12 the plants had increased fully 24 cm. in length, were healthy and deep green in color. No antheridia and oogonia were present on this new growth, however. At the end of October the plants had died, but a new growth was beginning at the base of the old plants. No reproductive organs have been observed on this culture since June 28. The control grew slowly but did not fruit during the spring and summer.

SERIES A

Collected from under the ice in the park pond.....March 15
Grown on east window-sill in laboratory.....March 15–19
In east window under electric light daily from sunset to 1 a. m.
March 19–April 22
Grown In north window with only daylight illumination..May 4–Nov. 6

This culture consisted of three jars of plants brought indoors on March 15, at a time when the pond in the park was frozen over. As soon as the cultures were placed in the east window in the laboratory they began to grow. On March 19 two jars were placed under electric light, which was kept burning until 1 a. m. The vegetative growth was more rapid than in the third jar, the control. On March 30, eleven days later, antheridia and oogonia appeared in abundance in the two jars which had been under the electric light, while there was no indication of fruiting in the control. On April 22 the experiment was terminated. Photographs of typical plants made on April 13 are shown in FIG. 3, PLATE 11.

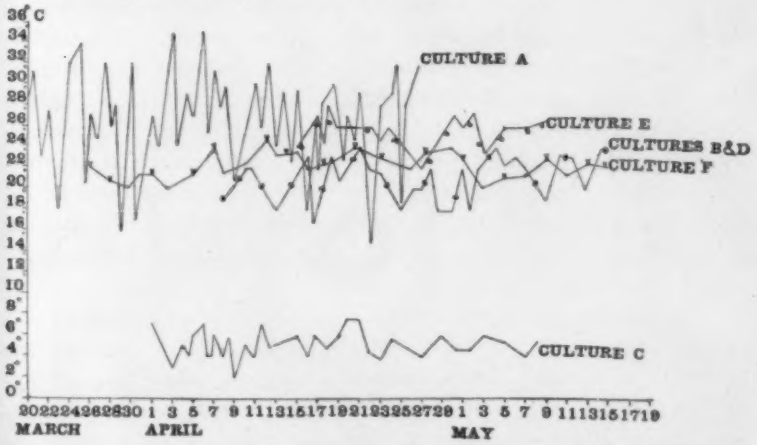
The temperature at which this culture was grown was 15° to 35° C., as shown in CHART 1. In some periods the daily range was as much as eighteen degrees. This wide daily variation in temperature may have been in part the cause of the abortion of the oogonia and the subsequent etiolation of the plants. As the experiment continued, the plants became light green in color, and by the end of April the basal nodes, internodes and leaves were almost white in color.

Owing to the advancing season this culture was receiving almost two hours additional daylight above that of the preceding one. On March 19 when this experiment was begun the daylight period was approximately 12 hours. When the fruiting was first observed on March 30, it had increased to 12½ hours,

and at the time of photographing the period of daylight was about 13½ hours. Since the artificial light was not turned on until sunset and then turned off at 1 a. m. there was a constant decrease in the period of electric illumination corresponding to the increase in daylight period.

The growth in length of the plants subjected to artificial illumination was generally greater and more rapid as noted, but etiolation developed as the experiment continued. The internodes were considerably longer than those of the control, and the leaves were shorter in proportion. By the end of April

CHART 1. Showing the temperature variations of the cultures of *Chara fragilis* throughout the period of experiments.



the control culture, left to itself, was overrun with filamentous algae, and at the end of May the *Chara* plants had completely disintegrated. The two cultures which had been subjected to the electric illumination were placed on a north window shelf away from the electric light on May 4. The subsequent growth of these plants was slow. A few antheridia and oogonia appeared on the new growth throughout the summer. FIG. 3, PLATE 13, shows three typical plants of Culture A photographed June 11. By the end of September one of these cultures was dead. Tips 10 cm. long from the remaining culture were transplanted to a pot of fresh sand loam. By November 6 a new vegetative growth was beginning, but no antheridia and oogonia were visible.

SERIES B

Collected from park pond.....March 26
Grown on shelf in south window.....March 26–April 8
Grown on shelf in south window under daily electric illumination from sunset to 10 p. m.....April 8–May 11
Grown on shelf in south window with only daylight illumination May 11–Nov. 6

Culture B consisted of two jars containing approximately two hundred shoots which had been brought indoors on March 26 and kept in a south window where the direct sunlight fell on the jars from 9 a. m. until 4 p. m. The control cultures were placed beside the two jars. The intensity of illumination in the early morning and late afternoon when the sun was not shining on the cultures, was measured with a photometer and found to be approximately 50 foot-candles. The electric light supplied was the same as in the preceding cultures, with the exception that it was turned off at 10 p. m. Antheridia and oogonia appeared four days after the artificial illumination was started. Photographs were made 33 days after the first reproductive organs appeared. FIG. 3, PLATE 12 shows the extent of fruiting at that time. The length of day, in the meantime, had increased one hour and ten minutes.

The production of antheridia and oogonia in this culture was not as abundant as in the preceding one. About half of the plants had formed no reproductive organs when the photographs were made. The vegetative growth, however, was striking. In nature, according to Migula, *Chara fragilis* does not reach much more than 75 cm. in length. In one jar of this culture, however, the plants attained a length of over a meter. The internodes were approximately 10 cm. long, but less than normal in diameter. All plants in the culture grew well during the summer. There was no apparent etiolation such as was so characteristic of the preceding culture. Fruiting throughout the summer was moderate. On September 12, tips of the plants about 10 cm. long were cut off and transplanted. By October 19 these transplanted tips had grown to a length of 30 cm., and of the hundred or more plants in the culture three showed a few antheridia and oogonia. On November 6, the plants were growing vigorously with occasional reproductive organs in eight of the plants. The stock culture from which

the tips were taken put out new branches after being pruned and grew rapidly. A few antheridia and oogonia were present on the new growth on November 6.

The temperature of the water of this culture was more constant than that of Culture A. The range of temperature extended between 16° and 25° C., and the variation within any 24 hours was not over nine degrees, as shown in CHART 1. This fairly low temperature appears to be the most favorable for continuous growth of the plants.

SERIES C

Collected from park pond.....March 26
Grown in south window.....March 26–31
Transferred to glass case containing ice in west window and grown under all-night electric illumination.....March 31–May 7

The plants of this culture were of the same lot as those of Culture B, collected at the same time and kept in a south window under the same conditions. On March 31 one jar with a control was transferred to a glass case in a west window where there was direct sunlight from 3 p. m. until sunset. The dimensions of the case were 50 × 60 × 100 cm. The sides and top were of thin clear glass that allowed full passage of light. Fifty pounds of ice was placed between the two jars each day. The temperature did not go above 7° C. throughout the experiment, and on several days it ran down as low as 2° C. The illumination in the glass case in the early forenoon was about 25 foot-candles. Electric illumination was continuous throughout the night with the light bulb in the same position as in the preceding experiments. On April 15, seven days after the all-night illumination was begun, antheridia and oogonia appeared.

The vegetative growth in this case during the time of the experiment was hardly noticeable. There was no elongation of the internodes, and scarcely any new leaves were formed. The antheridia and oogonia grew slowly, and at the time of photographing, May 28, were only one-fourth full size. FIG. 2, PLATE 12 shows a typical plant of this culture beside a control plant. The length of day for the period of this culture had increased about three hours and forty minutes beyond that for cultures o and A. Culture C was receiving several hours additional daylight and correspondingly less electric illumination.

When the refrigeration and electric illumination were discontinued the plants began to grow rapidly, and by the middle of June the plants were 18 cm. long, with abundant antheridia and oogonia.

SERIES D

Collected from park pond.....March 26
Grown in south window.....March 26–April 8
Grown in south window under two hours electric illumination in addition to daylight.....April 8–May 7
Grown in south window with only daylight illumination..May 7–Nov. 6

Culture D consisted of three jars of about 500 plants collected at the same time as those of Cultures B and C. They were kept alongside of Culture B in a south window in the laboratory and had the same temperature and light conditions up to April 8. On this date two of the jars were placed under electric illumination each night until 8:30 p. m., while the third jar was kept as a control. Antheridia and oogonia appeared on April 17, nine days after the experiment was begun. No reproductive organs were formed in the control culture. FIG. 1, PLATE 12 shows the extent of fruiting in contrast to that attained by Culture B in FIG. 1, B, PLATE 12.

The temperature conditions and length of day for the period of this culture were the same as for Culture B. The vegetative growth of the plants was rapid and vigorous throughout. By the middle of June the plants were about 55 cm. long and of a deep green color. The internodes were longer than those of plants growing in nature. No etiolation was evident.

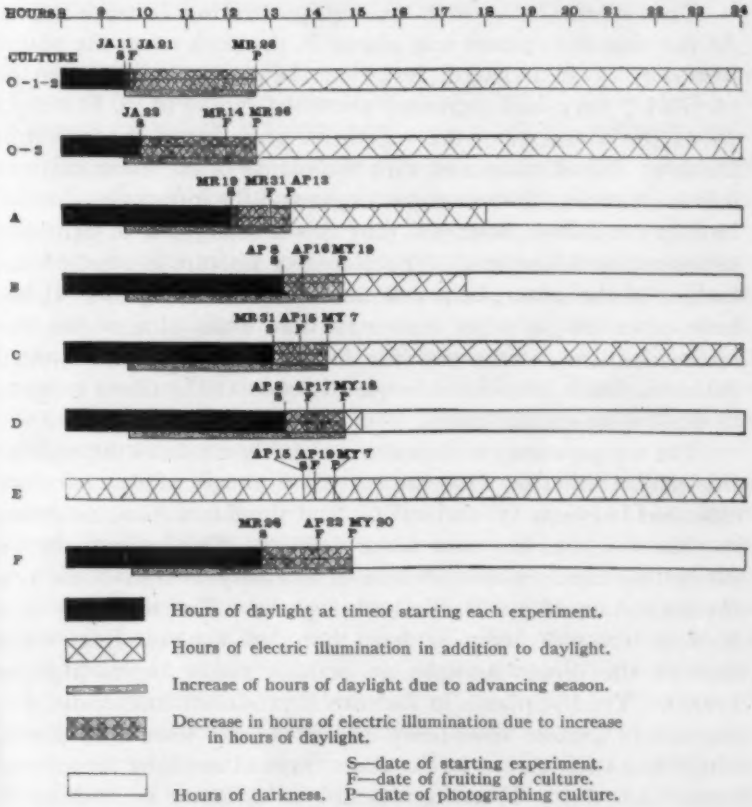
Culture D continued in a healthy growing condition throughout the summer and fall, but fruited only sparingly. The plants were approximately .75 meter long by September 12. On that date one of the cultures was pruned, and about fifty tips 10 cm. long were transplanted to a new jar with fresh soil, thus making four cultures in all. The transplanted tips as well as the pruned plants immediately began to grow. The other jars also showed vigorous growth. By October 19 the new growth in all the cultures was about 15 cm., and by November 6 most of the plants had abundant antheridia and oogonia.

SERIES E

Collected from park pond.....March 26
Grown in south window.....March 26–April 15
Grown in photographic dark room under electric illumination.April 15–May 7
Grown in east window.....May 7–Nov. 6

Culture E consisted of a jar of about one hundred and fifty shoots collected at the same time as those of Cultures B, C and D. It had been kept in the south window of the laboratory under the same light and temperature conditions as the three preceding cultures up to April 15. On that date the jar was transferred to a photographic dark room. The plants were il-

CHART 2. Summary and comparison of cultures of *Chara fragilis*.


HOURS
CULTURE
Hours of daylight at timeof starting each experiment.
Hours of electric illumination in addition to daylight.
Increase of hours of daylight due to advancing season.
Decrease in hours of electric illumination due to increase in hours of daylight.
Hours of darkness.
S—date of starting experiment.
F—date of fruiting of culture.
P—date of photographing culture.

luminated with electric light continuously from April 15 to May 7, receiving no daylight whatever. On April 19, four days after starting the experiment, rudimentary antheridia and oogonia were visible. By the end of the month they were full-sized and had begun to drop off and disintegrate. The production of antheridia and oogonia was more abundant than in any of the preceding cultures. Practically every leaf axil was full

of reproductive organs; whereas in the other cultures the antheridia and oogonia were developed only at the four uppermost nodes. None of the oogonia, however, developed mature oospores. The appearance of the antheridia and oogonia was normal in every respect. Motile antherozoids were produced in abundance, and the eggs were completely filled with large starch grains.

The vegetative growth was quite striking in appearance. At the time the culture was placed in the dark room the plants were only 15 cm. in length, but when the photographs were made on May 7 they had increased about 10 cm. FIG. 3, PLATE 12 shows the character of the vegetative growth and the extent of fruiting. When compared with the plants of the other cultures it is evident that the leaves are longer and the internodes shorter. It does not follow, however, that this is solely due to light and temperature differences. The plants of Culture E reached the surface of the water, bent over and continued to grow. It has been observed in other instances that when this occurs the internodes are shorter and the leaves longer than in normal cultures. Such behavior is frequently observed in *Chara* growing in shallow ponds and lakes.

The temperature of the water was fairly constant throughout the period of this experiment. The range of temperature extended between 23° and 27° C., and the fluctuation, as shown in CHART 1, was not over three degrees. This culture throws interesting light on the relation of intensity of illumination to the formation of antheridia and oogonia. The intensity of a 60-watt 120-volt lamp is less than ten foot-candles, while that of the direct sunlight at midday might be as high as 12,000. Yet the plants in Culture E produced antheridia and oogonia in greater abundance and in shorter time than plants which had been grown in the direct rays of sunlight for several hours each day. These results are in harmony with those of Garner and Allard, who found that the soy bean grown under one-fourth and one-third natural light intensity matured and produced seed in the same length of time as plants which had been grown in full sunlight. Klebs secured somewhat similar results with *Vaucheria* when the cultures were shaded. Using artificial light, he found zoospore and gamete production to be more abundant in an illumination equivalent to forty foot-candles than in higher intensities.

On May 12 Culture E was removed from the dark room to an east window in the laboratory. There it continued to grow slowly all summer, producing numerous antheridia and oogonia on the new growth. By September 12 the plants were about .75 meter in length. The culture was transferred to a clean jar on that date; and from that time to November 6 it grew steadily, until the plants were curled around in the jar several times. Antheridia and oogonia were abundant on all plants.

SERIES F

Collected in park pond.....March 26
Grown in full sunlight in a greenhouse at 22°–25° C.....March 26–May 18
Grown in full sunlight in a greenhouse.....May 18–Nov. 6

Since the response of *Chara fragilis* to increased periods of illumination in the laboratory was so direct, a series of cultures was run to determine the results of growing cultures in battery jars in a greenhouse where the plants would receive sunlight from sunrise to sunset during the spring months. On March 26, six large cultures with approximately 200 plants in each jar were started in a room in a greenhouse on which the direct rays of the sun fell the greater part of the day. The plants began to grow vigorously as soon as they were planted, but considerable difficulty was encountered in preventing them from being overrun by algae. The cultures were cleaned, and fresh water added several times, but by the end of May all but three of the cultures were dead. In the meantime, antheridia and oogonia appeared in the three remaining cultures on April 22. The length of day, reckoned from sunrise to sunset, at the time of fruiting was thirteen hours and fifty-five minutes. The temperature in the greenhouse in which Series F was grown was fairly constant and high. The variation was between 22° and 25° C. The growth at this temperature is rapid, but the plants become very slender and deteriorate in a short time. FIG. 1, PLATE 13 shows the variation of fruiting in different plants in these cultures on May 18.

On May 18, the three cultures were removed to a room with lower temperature. The plants continued to grow and fruit vigorously throughout the summer, and by September 12 some of the plants were .75 meter in length, with antheridia and oogonia in as many as six whorls of leaves. A large number of mature oospores were present on the plants. On September 12 the

cultures were cleaned and fresh water added. The growth from that time to November 6 was about 6 cm. A few antheridia and oogonia appeared on the new growth.

It is apparent from these results that a relatively long daily period of illumination is necessary to initiate the process of sexual reproduction in *Chara fragilis* in the spring months. The limit of the most favorable illumination period has not been determined, but it apparently falls beyond twelve hours.

SERIES G

Collected from the park pond.....March 19
Grown in south window of laboratory.....March 19–May 7

The great sensitiveness of these reactions to light is suggested by the record of three cultures growing in a south window of the laboratory. These cultures were started on March 19 and had the same temperature conditions as Series B and D. About 50 feet across from this window was a building with windows generally lighted as late as 11 p. m. This light was sufficiently intense to enable a person to read coarse print at the window where the cultures were standing. The reaction to this diffuse light was quite striking. On April 15, twenty-six days after the cultures were started, antheridia and oogonia appeared. In view of the results of other cultures like A, B, C, D and E, where an intensity of illumination of only a few foot-candles was sufficient to induce sexual reproduction, it seems probable that even this diffuse light had an appreciable effect in favoring the formation of antheridia and oogonia.

CONCLUSIONS

These experiments as noted were started mainly for the purpose of obtaining material for cytological study. It is apparent, however, that the sexual reproduction of *Chara fragilis* under the conditions described is in a considerable degree photoperiodic. The occurrence of the reaction is also independent, within a certain range, of the intensity of the illumination. These limits have not yet been determined, but an illumination of less than ten foot-candles was found sufficient under the conditions given to induce the formation of antheridia and oogonia in four days. Growing at the bottom of ponds and lakes as *Chara* is generally found, the illumination is mostly from above, and

relatively little lateral light reaches the plants. When grown in battery jars in the laboratory, however, the plants may receive light equally from all sides. As to the effects of intensity, it remains to be tested whether there is a quantitative difference in the response.

Under the conditions of my experiments temperature, within the minimum and maximum limits for vegetative growth, did not appear to be a primary factor in bringing out the tendency to reproduction. It does, however, have a marked effect on the rapidity of development of the antheridia and oogonia. In only one case, Series A, did the control culture form reproductive organs. Yet the controls had the same temperature conditions as the other cultures. Under the conditions given the formation of antheridia and oogonia can be induced at a temperature as low as 2° C. with continuous illumination. With my material and conditions the optimum temperature for continuous vegetative growth and gamete production varied between 18° and 25° C. At a constant temperature over 32° the plants soon deteriorated and died. These limits, however, are set only tentatively. Further experiments are necessary for definite proof.

It is not certain whether the failure of the eggs in most cases to develop into mature oospores is due to the unnatural conditions of the experiments or whether it is inherent in this particular form of *Chara fragilis*. This question remains to be studied in connection with observations on plants growing in nature. Culture 0–3 formed an abundance of mature oospores. The temperature for this culture was not as high as in the other experiments, and the out-of-doors conditions were perhaps more closely simulated. It is not clear whether the resulting sterility was due to the incompatibility of the gametes or to the abortion of the fertilized eggs.

SUMMARY

Under the conditions of the experiments described, length of day appears to be a primary factor in inducing the formation of antheridia and oogonia in *Chara fragilis*. A few hours of artificial illumination in addition to the daylight is sufficient to induce the development of antheridia and oogonia in mid- and late winter, whereas in nature *C. fragilis* fruits only from June to September, according to Migula (25) and Robinson (30).

Within wide limits the response is not dependent on the intensity of illumination under the conditions observed. A continuous illumination of less than ten foot-candles is sufficient to induce the development of antheridia and oogonia within four days.

Growth under artificial illumination, as used in my experiments, led to the lengthening of the internodes, shortening of the leaves, reduced branching, etiolation, and a general spindling habit in the plants. Very few of the eggs developed into mature oospores.

Under the conditions studied, the temperature, within the minimum and maximum limits for vegetative growth, is apparently a secondary factor in determining the production and functional activity of antheridia and oogonia in *Chara fragilis*.

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Explanation of plates 11–13

PLATE 11

FIG. 1. Typical plant of Cultures 0–1 and 0–2 made 65 days after antheridia and oogonia first appeared, × 3.

FIG. 2. Typical plant of Culture 0–3 made 70 days after fruiting began, natural size.

FIG. 3. Typical plant of Culture A made 14 days after antheridia and oogonia first appeared, × 2.

FIG. 4. Typical control plant of Culture A, × 3½.

PLATE 12

FIG. 1. Typical plants of Cultures D and B made 34 days after antheridia and oogonia were first formed, × 1¼.

FIG. 2. Typical plant of Culture C and its control made 50 days after fruiting began, × 2.

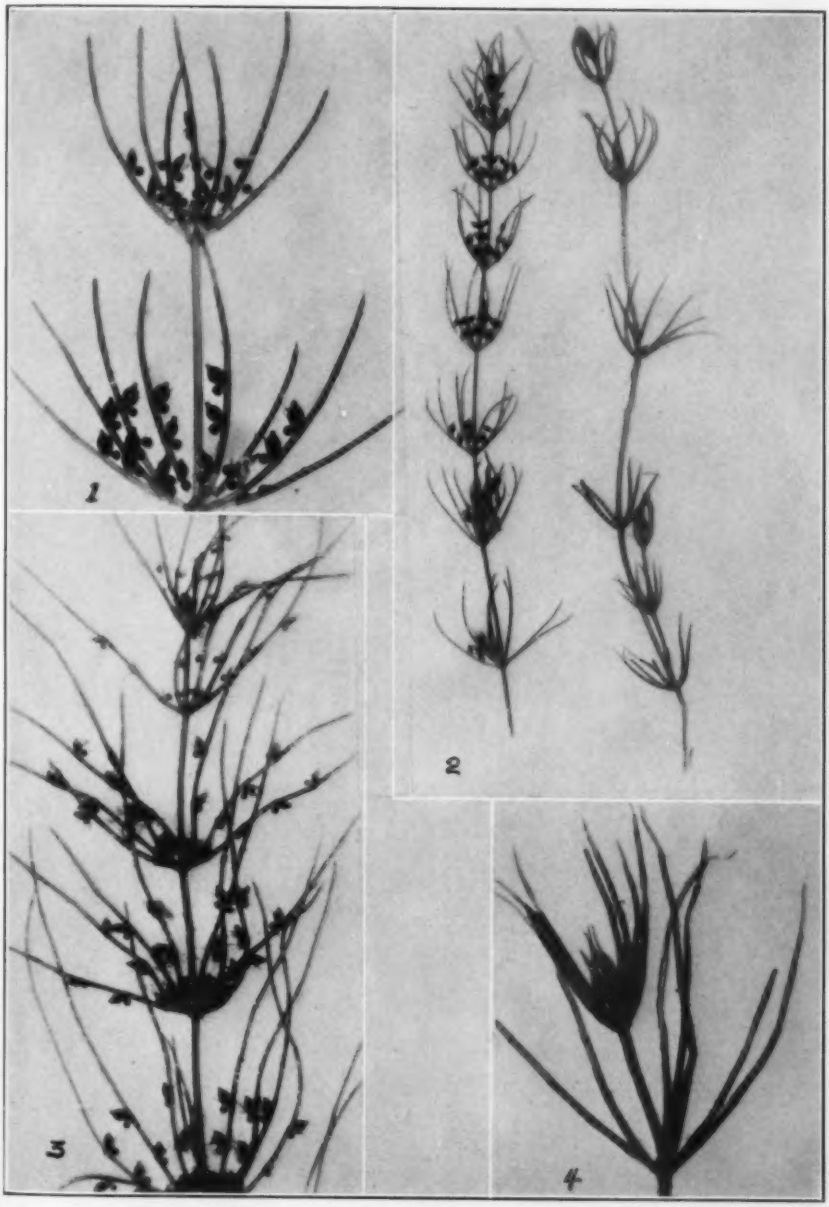
FIG. 3. Typical plant of Culture E made 27 days after fruiting began, × 2.

PLATE 13

FIG. 1. Typical plant of Culture F made 47 days after the culture was started, natural size.

FIG. 2. A typical distal node of plants in the control of Culture A after it had been left uncovered in the laboratory light 22 days, × 2.

FIG. 3. Typical plants of Culture A made two months after the experiment was terminated, × 1¼.



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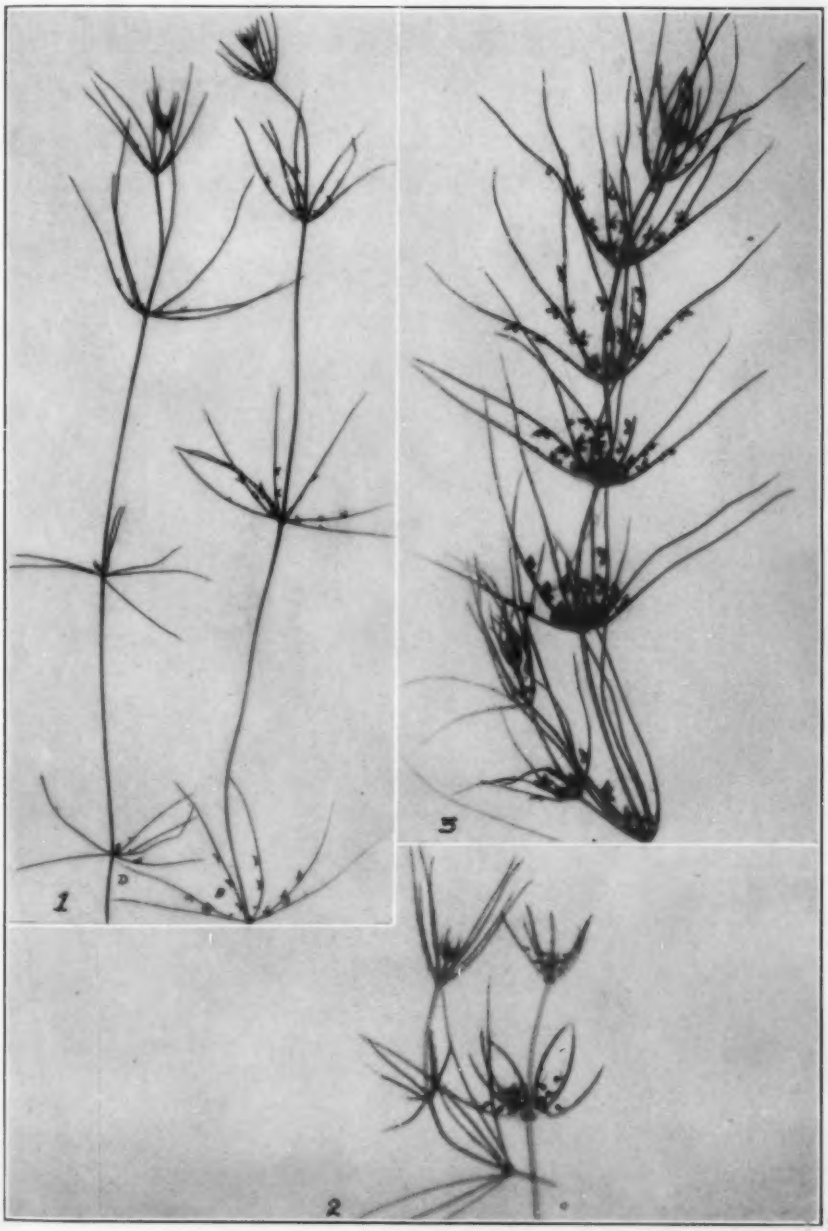

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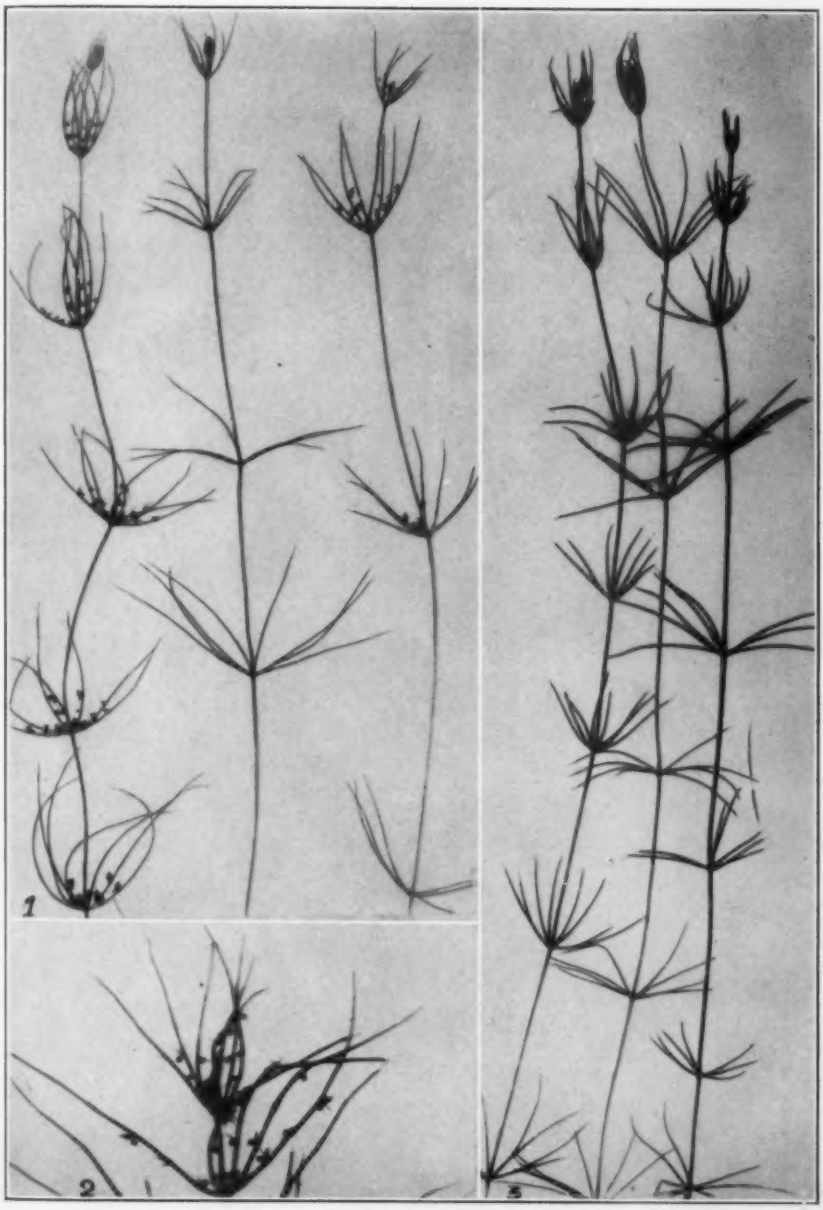

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INDEX TO AMERICAN BOTANICAL LITERATURE

1924

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